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Citral derived amides as potent bacterial NorA efflux pump inhibitors

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ABSTRACT

Monoterpene citral and citronellal have been used as starting material for the preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid amides and 9-formyl-5-methyl-deca-2,4,8-trienoic acid amides. The amides on bioevaluation as efflux pump inhibitors (EPIs) against *Staphylococcus aureus* 1199 and NorA overexpressing *S. aureus* 1199B bacteria resulted in the identification of several of these as potent EPIs. Many of these amides have been shown to possess potency higher or equivalent to known EPIs such as reserpine, verapamil, carsonic acid, and piperine. In this communication, we report a convenient synthesis of alkenyl amides, their bioevaluation and identification as efflux pump inhibitors against *S. aureus*.
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1. Introduction

Due to the emergence of multi-drug resistant (MDR) pathogenic bacteria, the management of the bacterial infections has become a daunting task. The MDR phenomenon[1,2] found in many species of bacterial, fungi, and tumor cell[3] is shown to occur mainly through three mechanisms namely target modification,[4–9] antibiotic inactivation,[10–12] or default of its accumulation within the cell and is responsible for exporting drugs from cells resulting in a low ineffective concentration of the drug.[13] MDR transporters recognize many structurally unrelated substrates and their presence may be there as a part of the detoxifying mechanisms in xenobiotics.[14]

Among several MDR transporters encompassing Gram-positive and Gram-negative bacteria, MDR pumps such as NorA transporter[15] (member of the major facilitator family-MF family), considered to be one of the major contributors toward drug effluxing, contribute to the resistance of *Staphylococcus aureus* to antibiotics such as ciprofloxacin by promoting their active extrusion from the cell.[16] To combat the menace of drug resistance, development of new drugs through rational drug design is a requirement that pharmaceutical companies and drug research centers need to address. However, an alternative approach that may come to stay in future is the identification of molecules that can interfere or decrease the effectiveness of efflux pumps and restore the activity of the drug molecule/s. Combination of antiinfective amoxicillin with antiinfective resistance inhibitor clavulanic acid is a pointer in that direction.[17] Therefore, development of clinically useful inhibitors that decrease the effectiveness of efflux pumps would represent a significant advance to provide successful treatment of multi-drug resistant conditions. Efforts to develop potent and clinically competent EPIs are being explored both from natural sources and through bioevaluation of synthetic chemical libraries,[19–30] and some interesting molecules have come up which are in the process of being developed as EPI's.[17,18]

In continuation of our research activities directed toward the development of EPIs involving natural as well as synthetic molecules, we have demonstrated the efficacy of piperine, a major constituent of *Piper nigrum*, as potent efflux pump inhibitor capable of reducing the MIC of ciprofloxacin-resistant mutant strain of *S. aureus* as well as methicillin resistance *S. aureus* (MRSA).[31] In addition to piperine, we have screened a library of piperine mimics and identified several of them as potent EPIs that showed fourfold reduction in MIC of ciprofloxacin at 6.25 μg/mL concentration of the EPIs.[32,33] In this communication, we report a convenient synthesis of alkenyl amides, their bioevaluation and identification as potent efflux pump inhibitors against *S. aureus* 1199 and NorA overexpressing *S. aureus* 1199B.

2. Results and discussion

Amides from both the natural and synthetic sources constitute an important class of compounds manifested with several biological activities that find their use as therapeutic agents in the treatment of various ailments or in agriculture as agrochemicals and in other fields.[34–39] An important property associated with aromatic

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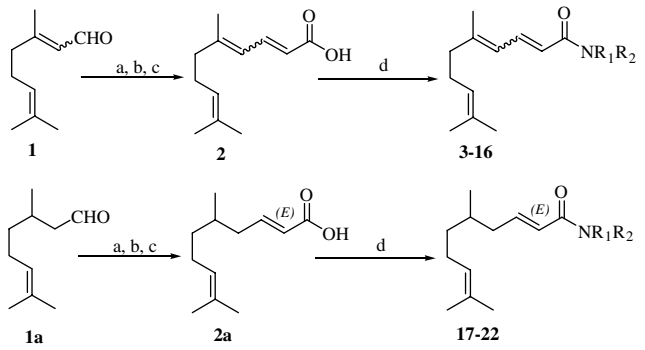


Scheme 1. Reagents: (a) $P(Ph)_3/BrCH_2COOEt/NaH/benzene$; (b) NaOH/MeOH; (c) HCl; 82–88%; (d) $SOCl_2/DCM$; NHR_1R_2/C_6H_6. NR_1R_2 = piperidine (**3**), benzylamine (**4**), *n*-octylamine (**5**), D-2-aminobutanol (**6**), L-2-aminobutanol (**7**), *o*-anisidine (**8**), *p*-anisidine (**9**), aniline (**10**), pyrrolidine (**11**), isopropyl amine (**12**), *o*-toluidine (**13**), morpholine (**14**), isobutylamine (**15**), diisopropylamine (**16**), piperidine (**17**), benzylamine (**18**), *n*-octylamine (**19**), D-2-aminobutanol (**20**), L-2-aminobutanol (**21**), and *o*-anisidine (22).

amides has been their recognition as potent efflux pump inhibitors of bacteria to check the emergence of multi-drug resistance offered by the microorganisms.[40–43]

Contrary to aromatic amides, alkyl/alkenyl/alkynl amides have not been explored as EPIs, but are reported to have several other biological properties, which include insecticidal, larvicidal, sialogogue, and antitumor activities.[44–49] It was, therefore, thought worthwhile to explore the possibility of preparing compounds belonging to the above-mentioned class and subject them to bioevaluation as efflux pump inhibitors particularly against NorA overexpressing *S. aureus* 1199B.

In this direction, we chose citral (*E*/*Z* ratio 77:23) (**1**) and citronellal (**1a)** as starting materials because of their easy availability, natural abundance, and low cost. The compounds **1** and **1a** were subjected to three-step reaction sequence to give 5,9-dimethyl-deca-2,4,8-trienoic acid amides (**3**–**16**) and 5,9-dimethyl-deca-2,8-dienoic acid amides (**17**–**22**), respectively, in moderate yields (Scheme 1).

These 5,9-dimethyl-deca-2,4,8-trienoic acid amides (**3**–**16**) and 5,9-dimethyl-deca-2,8-dienoic acid amides (**17**–**22**) in combination with antibacterial drug ciprofloxacin were subjected to bioevaluation for their possible role as EPI against *S. aureus* 1199 and NorA overexpressing *S. aureus* 1199B.The MIC of ciprofloxacin was determined against *S. aureus* 1199 and NorA overexpressing *S. aureus* 1199B in Muller–Hinton broth in the presence of increasing amounts of efflux pump inhibitors by broth checkerboard synergy method in a microtitre plates with twofold serial dilutions.[50] On bioevaluation of compounds **2**–**22** (used in combination with ciprofloxacin), seven compounds reduced the MIC of the antibiotic by fourfold as shown in Table 1.

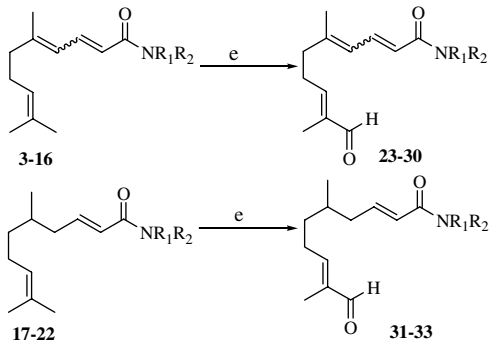
In an attempt to enhance the potentiating activity of these EPIs, we envisaged to bring in a modification in the amide molecules. The modification was effected by replacing C-9 methyl group of the amide molecules by a formyl group through oxidation by selenium dioxide to get modified molecules namely 9-formyl-5-methyl-deca-2,4,8-trienoic acid amides (**23**–**30**) and 9-formyl-5-methyl-deca-2,8-dienoic acid amides, respectively (**31**–**33**) (Scheme 2).

Bioevaluation of these modified molecules revealed the potentiation of the ciprofloxacin activity with most of the compounds exhibiting fourfold reduction in the MIC of ciprofloxacin as shown in Table 2. However, hydrogenation of double bond of some of the potent molecules (**5**, **8**, **23**, **24**, and **26**) to get partially saturated molecules (saturation of double bond/s conjugated to amide carbonyl) (Scheme 3) led to decrease in the potentiation of the antiinfective as shown in Table 2.

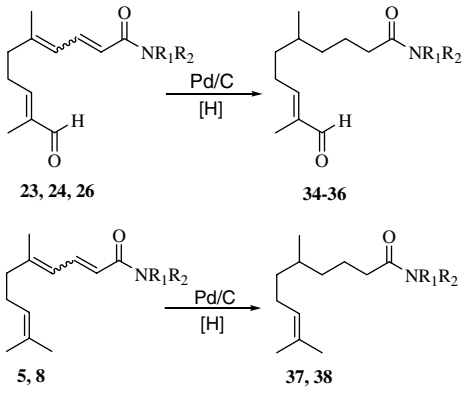
Table 1
Potentiation of ciprofloxacin MIC on *S. aureus* 1199B and *S. aureus* 1199 by synthetic compounds

Entry	MEC* of EPI	*S. aureus* 1199 B MIC** of Cipro (μg/mL)			*S. aureus* 1199 MIC** of Cipro (μg/mL)		
		Without EPI	With EPI	Fold reduction	Without EPI	With EPI	Fold reduction
2	50	8	8	0	0.25	0.25	0
3	12.5	8	4	2	0.25	0.12	2
4	25	8	2	4	0.25	0.12	2
	12.5	8	4	2	0.25	0.25	0
5	25	8	2	4	0.25	0.06	4
	12.5	8	4	2	0.25	0.12	2
6	25	8	2	4	0.25	0.06	4
7	25	8	2	4	0.25	0.12	2
8	25	8	2	4	0.25	0.12	2
9	50	8	8	0	0.25	0.25	0
10	50	8	8	0	0.25	0.25	0
11	50	8	8	0	0.25	0.25	0
12	50	8	8	0	0.25	0.25	0
13	50	8	8	0	0.25	0.25	0
14	50	8	8	0	0.25	0.25	0
15	50	8	8	0	0.25	0.25	0
16	25	8	2	4	0.25	0.12	2
17	12.5	8	4	2	0.25	0.25	0
18	50	8	2	4	0.25	0.06	4
19	50	8	8	0	0.25	0.25	0
20	50	8	8	0	0.25	0.25	0
21	50	8	8	0	0.25	0.25	0
22	12.5	8	4	2	0.25	0.25	0
Reserpine	25	8	2	4	0.25	0.12	2
Carsonic acid	25	8	4	2	0.25	0.12	2
Verapamil	50	8	4	2	0.25	0.12	2
Piperine	50	8	4	2	0.25	0.12	2

No antibacterial activity of EPIs was observed at 100 μg/mL that was the highest concentration tested.
For determining potentiation of ciprofloxacin, EPI was tested at concentration range of 50–0.8 μg/mL.
* MEC, minimum effective concentration.
** MIC, minimum inhibitory concentration.



Scheme 2. Reagents: (e) SeO_2/AcOH; 68–78%. NR_1R_2 = piperidine (**23**), benzylamine (**24**), *n*-octylamine (**25**), D-2-aminobutanol (**26**), L-2-aminobutanol (**27**), pyrrolidine (**28**), morpholine (**29**), diisopropylamine (**30**), *o*-anisidine (**31**), *n*-octylamine (**32**), and D-2-aminobutanol (**33**).



Scheme 3. Reagents: NR_1R_2 = piperidine (**34**), benzylamine (**35**), D-2-aminobutanol (**36**), *n*-octylamine (**37**), and *o*-anisidine (**38**).

The above studies revealed that unsaturation apparently seems to be an important factor responsible for drug potentiation, which is also the case with aromatic pentadienoic acid amides (earlier prepared and bioevaluated by us) wherein saturation of diene side chain show lowering of efflux pump inhibitory activity.[32] In the present study, we have been able to identify several potent molecules that exhibited better or equivalent potentiating activity when compared with known efflux pump inhibitors such as reserpine, verapamil, and piperine shown in Tables 1 and 2. The studies also show the effect of saturation or modification of the amide molecules on the overall potentiation activity of these synthesized molecules.

The inhibitory mechanism of the compounds was confirmed by efflux inhibition assay using ethidium bromide as substrate of NorA and compound **26** as the most potent inhibitor. Since ethidium bromide fluorescences only when it is bound to nucleic acids inside cells, there was a rapid decrease in fluorescence due to NorA mediated ethidium bromide efflux. Results presented in Figure 1 are the averages from triplicate measurements. As shown in Figure 1, only the control cells without EPIs extruded ethidium bromide, resulting in a significant decrease in fluorescence over the time of the assay. In the presence of each EPIs, loss of fluorescence was significantly reduced, reflecting a strong interference of ethidium bromide efflux by EPIs.

3. Conclusion

A convenient synthesis of a novel class of 5,9-dimethyl-deca-2,4,8-trienoic acid amides, 5,9-dimethyl-deca-2,8-dienoic acid amides, 9-formyl-5-methyl-deca-2,4,8-trienoic acid amides, and 9-formyl-5-methyl-deca-2,8-dienoic acid amides is reported for the first time in the present study. In combination with ciprofloxacin, their role as EPIs has been established. Seventeen out of 38 compounds potentiated the activity of the antibacterial

Table 2
Potentiation of ciprofloxacin MIC on *S. aureus* 1199B and *S. aureus* 1199 by synthetic compounds

Entry	MEC* of EPI	*S. aureus* 1199 B MIC** of Cipro (μg/mL)			*S. aureus* 1199 MIC** of Cipro (μg/mL)		
		Without EPI	With EPI	Fold reduction	Without EPI	With EPI	Fold reduction
23	25	8	2	4	0.25	0.25	0
	6.25	8	4	2	0.25	0.12	2
24	25	8	2	4	0.25	0.12	2
	12.5	8	4	2	0.25	0.25	0
25	25	8	2	4	0.25	0.12	2
	12.5	8	4	2	0.25	0.12	2
26	25	8	2	4	0.25	0.06	4
	6.25	8	4	2	0.25	0.12	2
27	50	8	8	0	0.25	0.25	0
28	50	8	8	0	0.25	0.25	0
29	25	8	8	0	0.25	0.25	0
30	25	8	2	4	0.25	0.25	0
	12.5	8	4	2	0.25	0.25	0
31	25	8	2	4	0.25	0.25	0
32	25	8	2	4	0.25	0.25	0
33	50	8	8	0	0.25	0.25	0
34	50	8	8	0	0.25	0.25	0
35	50	8	8	0	0.25	0.25	0
36	50	8	8	0	0.25	0.25	0
37	50	8	8	0	0.25	0.25	0
38	50	8	8	0	0.25	0.25	0
Reserpine	25	8	2	4	0.25	0.12	2
Carsonic acid	25	8	4	2	0.25	0.12	2
Verapamil	50	8	4	2	0.25	0.12	2
Piperine	50	8	4	2	0.25	0.12	2

No antibacterial activity of EPIs was observed at 100 μg/mL that was the highest concentration tested.
For determining potentiation of ciprofloxacin, EPI was tested at concentration range of 50–0.8 μg/mL.
* MEC, minimum effective concentration.
** MIC, minimum inhibitory concentration.

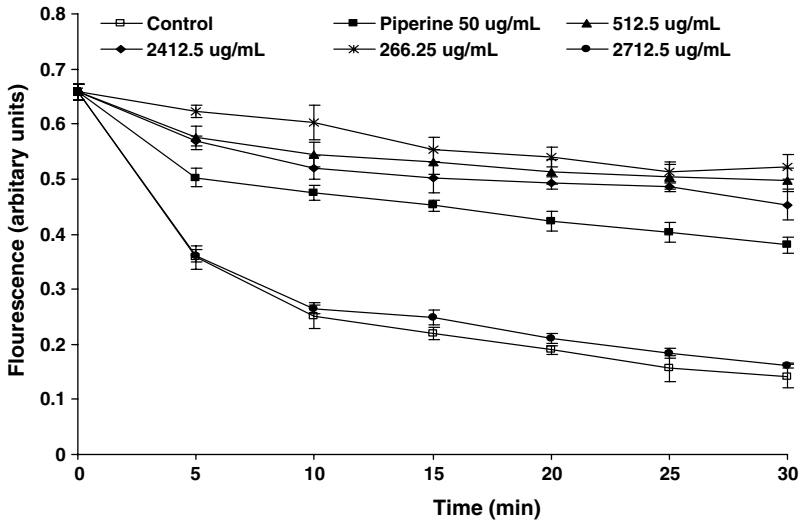


Figure 1. Ethidium bromide efflux inhibition assay from *S. aureus* SA 1199B cells. The cells were loaded with ethidium bromide and the efflux was allowed to occur in the absence of EPI (Control) or in the presence of EPIs. Each time point represents the mean $\log_{10} \pm$ SD of three readings.

agent ciprofloxacin by blocking the bacterial efflux pump in *S. aureus* 1199. The effect has been found more prominent in NorA overexpressing *S. aureus* 1199B. The mechanism of action of these compounds has been established by ethidium bromide fluorescence experiment (Fig. 1). The compounds **5–8** from 5,9-dimethyl-deca-2,4,8-trienoic acid group of amides, compound **18** from 5,9-dimethyl-deca-2,8-dienoic acid group of amides, compounds **23, 24, 25**, **26**, and **30** from 9-formyl-5-methyl-deca-2,4,8-trienoic acid amide group and compounds **31** and **32** from 9-formyl-5-methyl-deca-2,8-dienoic acid group of amides proved to be the most effective inhibitors. The potential clinical utility of these molecules as EPIs warrants further investigations.

4. Experimental

4.1. General chemistry methods

All reagents for chemical synthesis were obtained from Sigma–Aldrich. The starting material citral (a/b) was isolated from lemon grass oil. All the solvents used in reactions were distilled and dried before use. All reactions were monitored by TLC on 0.25 mm silica gel 60 F_{254} plates (E. Merck) using UV light, or ceric sulfate solution for detection of the spots. Silica gel 60–120 mesh was used for column chromatography. All NMR spectra were recorded on Bruker DPX 200 instrument using $CDCl_3$ as the solvent with TMS as internal standard. Chemical shift is expressed in δ (ppm) and coupling constants in Hertz. Mass spectra were recorded on ESI-esquire 3000 Bruker Daltonics instrument. IR spectra were recorded on Bruker Vector 22 instrument.

4.2. Synthesis

4.2.1. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid (2)[51,52]

To a stirring mixture of the ylide (55.58 g, 129.86 mmol) (prepared from ethyl bromoacetate and triphenylphosphine) in dry benzene (100 mL), sodium hydride (4.0 g) was added in small proportion. Then, citral **1** (20 g, 130 mmol) in dry benzene was added drop wise into the reaction mixture for 1 h. The reaction progress was monitored by TLC and on completion of the reaction, the contents were poured carefully in ethyl acetate to quench the excess of sodium hydride, followed by addition of water, the organic layer was separated out, and the aqueous layer extracted with ethyl acetate (3× 100 mL). The combined organic layer was washed with water (3× 50 mL), dried over anhydrous sodium sulfate and concentrated on a rotavapor under reduced pressure. The crude product was taken in 10% methanolic NaOH solution (200 mL) and the contents were refluxed for 6 h. The contents were cooled and extracted with ethyl acetate (3× 150 mL). The aqueous layer was washed with petroleum ether (50 mL) and acidified with 2 N HCl solution. The resulting precipitate was extracted with ethyl acetate (3× 100 mL), the organic layer washed with water (3× 150 mL), dried over anhydrous sodium sulfate and concentrated to give crude product, which on purification over silica gel column using pet.ether/ethyl acetate (8:2) as an eluent gave compound **2** (20.65 g, 82%). Anal. Calcd for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34%. Found: C, 74.21; H, 9.36%. MS (%) M^+ at *m*/*z* M^++1 195 (36), 149 (62), 124 (12), 111 (79), 81 (74), 67 (100). IR (neat). 3407, 2974, 2929, 1708, 1383, 1260, 1161, 1073, 814, 757, 592 cm^{-1}. ^1H NMR major isomer: δ 1.60 and 1.68 (3H each, s, =C(CH_3)$_2$), 1.90 (3H, s, =CCH_3), 2.16 (4H, br s, 2× CH_2), 5.09 (1H, br s, CH_2CH=), 5.75 (1H, d, *J* = 15.1 Hz, CH=C*H*CO), 6.03 (1H, d, *J* = 11.5 Hz C*H*CH=CHCO), 7.67 (1H, dd, *J* = 11.6 and 15.1 Hz, CHC*H*=CHCO).

The methyl ester of **2** was prepared in 97% yield by diazomethane as per literature method.[52,53] Anal. Calcd for $C_{13}H_{20}O_2$: C, 74.96; H, 9.68%. Found: C, 74.99; H, 9.70%. MS (%) M^+ at *m*/*z* 208 (30), 193 (28), 165 (24), 149 (52), 125 (100), 109 (18). IR (neat). 3463, 2974, 1725, 1437, 1376, 1271, 1168, 986, 740, 582 cm^{-1}. ^1H NMR major isomer: δ 1.54 and 1.60 (3H each, s, =C(CH_3)$_2$), 1.81 (3H, s, =CCH_3), 2.07 (4H, br s, 2× CH_2), 3.65 (3H, s, COOCH_3), 5.01 (1H, br s, CH_2CH=), 5.69 (1H, d, *J* = 15.2 Hz, CH=C*H*CO), 5.92 (1H, d, *J* = 11.7 Hz, C*H*CH=CHCO), 7.51 (1H, dd, *J* = 11.7 and 15.2 Hz, CHC*H*=CHCO).

The ethyl ester of **2** was prepared in 95% yield as per literature method.[52,54] Anal. Calcd for $C_{14}H_{22}O_2$: C, 75.63; H, 9.97%. Found: C, 75.65; H, 9.99%. MS (%) M^+ at *m*/*z* 222 (29), 193 (100), 149 (56), 122 (43), 109 (71). IR (neat). 2961, 2924, 1722, 1653, 1457, 1378, 1368, 1311, 1264, 1182, 1096, 1046, 983, 806 cm^{-1}. ^1H NMR major isomer: δ 1.29 (3H, t, *J* = 7.1 Hz, CH_2CH_3), 1.58 and 1.68 (3H each, s, =C(CH_3)$_2$), 1.89 (3H, s, =CCH_3), 2.15 (4H, br s, 2× CH_2), 4.19 (2H, q, CH_2CH_3), 5.09 (1H, br s, CH_2CH=), 5.76 (1H, d, *J* = 15.1 Hz, CH=C*H*CO), 5.98 (1H, d, *J* = 11.2 Hz, C*H*CH=CHCO), 7.56 (1H, dd, *J* = 11.2 and 15.1 Hz, CHC*H*=CHCO).

4.2.2. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid piperidide (3)

To dichloromethane (DCM) solution of **2** (0.5 g, 2.57 mmol, 30 mL) added freshly distilled thionyl chloride (0.53 mL) and re-

fluxed the contents for 1 h. The excess of thionyl chloride was removed on rotavapor under reduced pressure and the acid chloride reconstituted in DCM and to this added DCM solution of piperidine (0.5 mL) and stirred the contents for 30 min. The organic layer washed with water (2× 25 mL), dried over anhydrous sodium sulfate and concentrated on rotavapor under reduced pressure to give crude product, which on purification over silica gel using pet.ether/ethyl acetate (92:8) mixture as an eluent afforded pale yellow gummy mass **3** (0.6 g, yield 90%). Anal. Calcd $C_{17}H_{27}NO$: C, 78.11; H, 10.41; N, 5.36%. Found: C, 78.63; H, 10.45; N, 5.38%. MS (%) M^+ at *m*/*z* 261 (11), 246 (7), 206 (4), 193 (14), 179 (45), 178 (46), 112 (100), 109 (17), 84 (72), 83 (36), 81 (55), 80 (30). IR (neat). 3418, 2949, 2806, 2524, 1644, 1616, 1456, 1270, 1020, 554 cm^{-1}. ^1H NMR major isomer: δ 1.57 (6H, br s, –N–$CH_2(CH_2)_3$), 1.66 and 1.83 (3H each, s, =C(CH_3)$_2$), 2.10 (3H, s, =CCH_3), 2.30 (4H, br s, 2× CH_2), 3.37 (4H, br s, –N(CH_2)$_2$), 5.06 (1H, br s, CH_2CH=), 5.98 (1H, d, *J* = 11.4 Hz, C*H*CH=CHCO), 6.24 (1H, d, *J* = 14.6 Hz, CH=C*H*CO), 7.56 (1H, dd, *J* = 11.4 and 14.6 Hz, CHC*H*=CHCO).

4.2.3. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid benzyl amide (4)

This was prepared in 94% yield from **2** by the procedure as described for compound **3**. Anal. Calcd for $C_{19}H_{25}NO$: C, 80.52; H, 8.89; N, 4.94%. Found: C, 80.83; H, 8.91; N, 4.99%. MS (%) M^+ at *m*/*z* 283 (28), 255 (12), 206 (56), 149 (87), 134 (74), 124 (100), 123 (71), 121 (10), 106 (15), 83 (20), 77 (23), 73 (10), 72 (10), 69 (97) and 59 (51). IR (neat). 3287, 2967, 2925, 1653, 1541, 1453, 1280, 1177, 1081, 1029, 740 cm^{-1}. ^1H NMR major isomer: δ 1.60 and 1.67 (3H each, s, =C(CH_3)$_2$), 1.85 (3H, s, =CCH_3), 2.13 (4H, br s, 2× CH_2), 4.52 (2H, s, –NH–CH_2), 5.06 (1H, br s, CH_2CH=), 5.76 (1H, d, *J* = 14.7 Hz, CH=C*H*CO), 5.97 (1H, d, *J* = 11.7 Hz, C*H*CH=CHCO), 7.26–7.37 (5H, m, 5× Ar-H), 7.57 (1H, dd, *J* = 11.7 and 14.7 Hz, CHC*H*=CHCO).

4.2.4. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid *n*-octylamide (5)

This was prepared in 70% yield from **2** by the procedure as described for compound **3**. Anal. Calcd for $C_{20}H_{35}NO$: C, 78.63; H, 11.55; N, 4.58%. Found: C, 79.01; H, 11.57; N, 4.61%. MS (%) M^+ at *m*/*z* M^++1 306 (2), 263 (33), 221 (42), 203 (85), 193 (60), 179 (25), 153 (75), 138 (90), 129 (96), 91 (95), 46 (100). IR (neat). 3293, 2957, 2926, 1651, 1547, 1456, 1376, 979, 722 cm^{-1}. ^1H NMR major isomer: δ 0.89 (3H, t, *J* = 6.6 Hz –CH_2–CH_3), 1.12–1.24 (12H, m, –NH$CH_2(CH_2)_6$), 1.62 and 1.68 (3H each, s, =C(CH_3)$_2$), 1.89 (3H, s, =CCH_3), 2.16 (4H, br s, 2× CH_2), 3.18 (2H, m, –NH–CH_2), 5.10 (1H, br s, CH_2CH=), 5.80–6.02 (2H, m, C*H*CH=C*H*CO), 7.47 (1H, dd, *J* = 11.5 and 14.9 Hz, CHC*H*=CHCO).

4.2.5. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid amide of D-2-aminobutanol (6)

This was prepared in 72% yield from **2** by the procedure as described for compound **3**. Anal. Calcd for $C_{16}H_{27}NO_2$: C, 72.41; H, 10.25; N, 5.28%. Found: C, 72.49; H, 10.26; N, 5.29%. MS (%) M^+ at *m*/*z* 265 (10), 248 (15), 193 (21), 186 (55), 149 (100), 138 (54), 119 (24), 100 (85), 88 (25). IR (neat). 3332, 2966, 2930, 2877, 1652, 1541, 1458, 1382, 1092, 1019, 671 cm^{-1}. ^1H NMR major isomer: δ 0.94 (3H, t, *J* = 7.2 Hz, CH_2–CH_3), 1.52, (2H, m, CH_2–CH_3), 1.62 and 1.66 (3H each, s, =C(CH_3)$_2$), 1.82 (3H, s, =CCH_3), 2.12 (4H, br s, 2× CH_2), 3.54–3.72 (3H, m, –NH–C*H*–CH_2–OH), 5.05 (1H, br s, CH_2CH=), 5.78 (1H, d, *J* = 11.7 Hz, C*H*CH=CHCO), 5.99 (1H, d, *J* = 14.8 Hz, CH=C*H*CO), 7.50 (1H, dd, *J* = 11.7 and 14.8 Hz, CHC*H*=CHCO).

4.2.6. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid amide of L-2-aminobutanol (7)

This was prepared in 72% yield from **2** by the procedure as described for compound **3**. Anal. Calcd for $C_{16}H_{27}NO_2$: C, 72.41; H, 10.25; N, 5.28%. Found: C, 72.44; H, 10.27; N, 5.33%. MS (%) M^+ at *m*/*z* 265 (7), 248 (5), 193 (25), 186 (62), 149 (100), 138 (71), 119 (19), 100 (90), 88 (20). IR (neat). 3431, 2967, 2930, 1688, 1634, 1414, 1231, 1161, 981, 756 cm^{-1}. ^1H NMR major isomer: δ 0.98 (3H, t, *J* = 7.3 Hz, CH_2–CH_3), 1.54, (2H, m, CH_2CH_3), 1.61 and 1.68 (3H each, s, =C(CH_3)$_2$), 1.84 (3H, s, =CCH_3), 2.15 (4H, br s, 2× CH_2), 3.61–3.78 (3H, m, –NHC*H*CH_2OH), 5.09 (1H, br s, CH_2CH=), 5.77 (1H, d, *J* = 11.2 Hz, C*H*CH=CHCO), 5.96 (1H, d, *J* = 14.5 Hz, CH=C*H*CO), 7.56 (1H, dd, *J* = 11.2 and 14.5 Hz, CHC*H*=CHCO).

4.2.7. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid *o*-anisidine amide (8)

This was prepared in 90% yield from **2** by the procedure as described for compound **3**. Anal. Calcd for $C_{19}H_{25}NO_2$: C, 76.22; H, 8.42; N, 4.68%. Found: C, 76.30; H, 8.42; N, 4.71%. MS (%) M^+ at *m*/*z* 299 (2), 284 (40), 271 (6), 241 (73), 198 (13), 186 (9), 150 (13), 122 (31), 108 (25), 107 (27), 95 (6), 80 (12), 74 (100). IR (neat). 3419, 3323, 2965, 2929, 1667, 1627, 1601, 1522, 1460, 1287, 1251, 1046, 787 cm^{-1}. ^1H NMR major isomer: δ 1.61 and 1.68 (3H each, s, =C(CH_3)$_2$), 1.89 (3H, s, =CCH_3), 2.15 (4H, br s, 2× CH_2), 3.88 (3H, s, Ar-OCH_3), 5.08 (1H, br s, CH_2CH=), 5.89–6.05 (2H, m, C*H*CH=C*H*CO), 6.84–7.04 (4H, m, 4× Ar-H), 7.64 (1H, dd, *J* = 11.4 and 14.6 Hz, CHC*H*=CHCO).

4.2.8. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid *p*-anisidine amide (9)

This was prepared in 88% yield from acid **2** by the procedure as described for compound **3**. Anal. Calcd for $C_{19}H_{25}NO_2$: C, 76.22; H, 8.42; N, 4.68%. Found: C, 76.24; H, 8.41; N, 4.74%. MS (%) M^+ at *m*/*z* M^++1 300 (12), 270 (24), 193 (34), 184 (40), 149 (75), 138 (100), 100 (32). IR (neat). 3331, 2972, 2929, 1717, 1660, 1603, 1511, 1341, 1244, 777 cm^{-1}. ^1H NMR major isomer: δ 1.60 and 1.68 (3H each, s, =C(CH_3)$_2$), 1.89 (3H, s, =CCH_3), 2.19 (4H, br s, 2× CH_2), 3.78 (3H, s, Ar-OCH_3), 5.12 (1H, br s, CH_2CH=), 5.97–6.03 (2H, m, C*H*CH=C*H*CO), 6.91–7.21 (4H, m, Ar-H), 7.43 (1H, dd, *J* = 11.8 and 14.8 Hz, CHC*H*=CHCO).

4.2.9. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid anilide (10)

This was prepared in 88% yield from **2** by the procedure as described for compound **3**. Anal. Calcd for $C_{18}H_{23}NO$: C, 80.26; H, 8.61; N, 5.20%. Found: C, 80.27; H, 8.63; N, 5.29%. MS (%) M^+ at *m*/*z* 269 (52), 227 (70), 200 (81), 199 (69), 186 (18), 185 (86), 177 (30), 123 (33), 109 (77), 74 (100). IR (neat). 3304, 2965, 2928, 1661, 1626, 1598, 1540, 1500, 1440, 1274, 1031, 755, 669 cm^{-1}. ^1H NMR major isomer: δ 1.61 and 1.68 (3H each, s, =C(CH_3)$_2$), 1.82 (3H, s, =CCH_3), 2.16 (4H, br s, 2× CH_2), 5.10 (1H, br s, CH_2CH=), 5.89 (1H, d, *J* = 11.4 Hz, C*H*CH=CHCO), 6.01 (1H, d, *J* = 14.7 Hz CH=C*H*CO), 7.07–7.15 (2H, d, *J* = 7.6 Hz, Ar-H), 7.27–7.38 (3H, m, Ar-H), 7.58 (1H, dd, *J* = 11.4 and 14.7 Hz, CHC*H*=CHCO).

4.2.10. Preparation 5,9-dimethyl-deca-2,4,8-trienoic acid pyrrolidide (11)

This was prepared in 91% yield from **2** by the procedure as described for compound **3**. Anal. Calcd for $C_{16}H_{25}NO$: C, 77.68; H, 10.19; N, 5.66%. Found: C, 77.88; H, 10.20; N, 5.71%. MS (%) M^+ at *m*/*z* 247 (64), 149 (93), 147 (70), 139 (25), 98 (18), 95 (6), 79 (4), 70 (13), 46 (100). IR (neat). 3403, 2972, 2877, 1620, 1449, 1377, 1165, 754 cm^{-1}. ^1H NMR major isomer: δ 1.61 and 1.67 (3H each, s, =C(CH_3)$_2$), 1.90 (3H, s, =CCH_3), 1.95 (4H, br s, –N–$CH_2(CH_2)_2$), 2.67 (4H, br s, 2× CH_2), 3.52 (4H, br s, –N–(CH_2)$_2$), 5.11 (1H, br s, CH_2CH=), 6.09 (1H, d, *J* = 11.8 Hz, C*H*CH=CHCO), 6.42 (1H, d, *J* = 14.6 Hz CH=C*H*CO), 7.54 (1H, dd, *J* = 11.8 and 14.6 Hz, CHC*H*=CHCO).

4.2.11. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid isopropyl amide (12)

This was prepared in 90% yield from **2** by the procedure as described for compound **3**. Anal. Calcd for $C_{15}H_{25}NO$: C, 76.55; H, 10.71; N, 5.95%. Found: C, 76.87; H, 10.73; N, 5.99%. MS (%) M^+ at *m*/*z* 235 (40), 180 (100), 179 (35), 166 (54), 152 (36) 151 (33), 136 (28), 96 (11), 82 (15), 68 (88). IR (neat). 3332, 2966, 2930, 2877, 1652, 1541, 1458, 1382, 1282, 1092, 1019, 671, 615 cm^{-1}. ^1H NMR major isomer: δ 1.30 (6H, d, *J* = 6.5 Hz, –NHCH(CH_3)$_2$), 1.62 and 1.67 (3H each, s, =C(CH_3)$_2$), 1.88 (3H, s, =CCH_3), 2.15 (4H, br s, 2× CH_2), 4.01 (1H, m, –NHC*H*), 4.98 (1H, br s, CH_2CH=), 5.89–6.05 (2H, m, *CH*CH=*CH*CO), 7.48 (1H, dd, *J* = 11.5 and 14.9 Hz, CHC*H*=CHCO).

4.2.12. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid *o*-toluidine (13)

This was prepared in 90% yield from **2** by the procedure as described for compound **3**. Anal. Calcd for $C_{19}H_{25}NO$: C, 80.52; H, 8.89; N, 4.94%. Found: C, 80.66; H, 8.91; N, 4.93%. MS (%) M^+ at *m*/*z* 283 (30), 198 (100), 191 (17), 190 (27), 177 (80), 166 (59), 160 (91), 138 (67), 107 (53), 93 (30), 92 (7), 83 (59), 69 (80), 55 (43). IR (neat). 3317, 2926, 2855, 1715, 1604, 1511, 1383, 1244, 1073, 1035, 829 cm^{-1}. ^1H NMR major isomer: δ 1.60 and 1.68 (3H each, s, =C(CH_3)$_2$), 1.89 (3H, s, =CCH_3), 2.15 (4H, br s, 2× CH_2), 2.29 (3H, s, Ar-CH_3), 5.09 (1H, br s, CH_2CH=), 5.82–6.03 (2H, m, *CH*CH=*CH*CO), 7.03–7.22 (4H, m, Ar-H), 7.59 (1H, dd, *J* = 11.6 and 15.2 Hz, CHC*H*=CHCO).

4.2.13. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid morpholide (14)

This was prepared in 90% yield from **2** by the procedure as described for compound **3**. Anal. Calcd for $C_{16}H_{25}NO_2$: C, 72.96; H, 9.57; N, 5.32%. Found: C, 72.97; H, 9.56; N, 5.34%. MS (%) M^+ at *m*/*z* 263 (30), 208 (54), 190 (34), 148 (41), 133 (14), 120 (63), 119 (38), 115 (61), 107 (76), 101 (92), 100 (100), 95 (14). IR (neat). 3386, 2927, 2857, 1643, 1439, 1378, 1115, 760 cm^{-1}. ^1H NMR major isomer: δ 1.61 and 1.67 (3H each, s, =C(CH_3)$_2$), 1.89 (3H, s, =CCH_3), 2.12 (4H, br s, 2× CH_2), 3.35 (4H, br s, –N–(CH_2)$_2$), 3.65 (4H, br s, –O–(CH_2)$_2$), 5.10 (1H, br s, CH_2CH=), 6.09 (1H, d, *J* = 11.7 Hz, *CH*CH=CHCO), 6.42 (1H, d, *J* = 14.6 Hz, CH=*CH*CO), 7.56 (1H, dd, *J* = 11.7 and 14.6 Hz, CHC*H*=CHCO).

4.2.14. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid isobutyl amide (15)[32]

This was prepared in 72% yield from **2** by the procedure as described for compound **3**. Anal. Calcd for $C_{16}H_{27}NO$: C, 70.06; H, 10.91; N, 5.62%. Found: C, 72.07; H, 10.92; N, 5.29%. MS (%) M^+ at *m*/*z* 249 (75), 234 (60), 206 (25), 191 (100), 162 (70), 129 (80), 93 (95), 53 (70). IR (neat). 3312, 2925, 2854, 1718, 1655, 1544, 1384, 1279, 1073, 981, 813 cm^{-1}. ^1H NMR major isomer: δ 0.84 (6H, d, *J* = 5.5 Hz, CH(CH_3)$_2$), 1.61 and 1.68 (3H each, s, =C(CH_3)$_2$), 1.71, (1H, m, *CH*(CH_3)$_2$), 1.85 (3H, s, =CCH_3), 2.08 (4H, br s, 2× CH_2), 3.17 (2H, t, *J* = 6.4 Hz, –NH–CH_2), 5.06 (1H, br s, CH_2CH=), 5.75 (1H, d, *J* = 10.2 Hz, *CH*CH=CHCO), 6.08 (1H, d, *J* = 14.9 Hz, CH=*CH*CO), 7.51 (1H, dd, *J* = 10.2 and 14.9 Hz, CHC*H*=CHCO).

4.2.15. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid diisopropylamide (16)

This was prepared in 96% yield from **2** by the same procedure as described for compound **3**. Anal. Calcd for $C_{18}H_{31}NO$: C, 77.92; H, 11.26; N, 5.05%. Found: C, 77.00; H, 11.29; N, 5.10%. MS (%) M^+ at *m*/*z* 277 (35), 234 (60), 191 (15), 176 (55), 148 (15), 122 (100), 107 (25), 92 (15). IR (neat). 2922, 2852, 1648, 1453, 1378, 1265, 1087, 821 cm^{-1}. ^1H NMR major isomer: δ 0.81 (12H, d, 2× CH(CH_3)$_2$), 1.64 and 1.71 (3H each, s, =C(CH_3)$_2$), 1.83 (3H, s, =CCH3), 2.25 (4H, br s, 2× CH_2), 4.12 (2H, m, 2× *CH*(CH_3)$_2$), 5.08 (1H, br s, CH_2CH=), 5.78 (1H, d, *J* = 10.3 Hz, *CH*CH=CHCO), 6.11 (1H, d, *J* = 15.0 Hz, CH=*CH*CO), 7.53 (1H, dd, *J* = 10.3 and 15.0 Hz, CHC*H*=CHCO).

4.2.16. Preparation of 5,9-dimethyl-deca-2,8-dienoic acid (2a)

This was prepared in 88% yield from **1a** by the procedure as described for compound **2**. Anal. Calcd for $C_{12}H_{20}O_2$: C, 73.43; H, 10.27%. Found: C, 73.46; H, 10.30%. MS (%) M^+ at *m*/*z* 196 (30), 152 (52), 126 (46), 112 (75), 83 (54), 69 (100). IR (neat). 2961, 2922, 1701, 1651, 1419, 1379, 1282, 1093, 984, 939, 686 cm^{-1}. ^1H NMR major isomer: δ 0.93 (3H, d, *J* = 6.8 Hz, CHCH_3), 1.08–1.17 (3H, m, CH_3CHCH_2), 1.58 and 1.64 (3H each, s, =C(CH_3)$_2$), 1.94–2.29 (4H, m, CH_2CHCH_2), 5.08 (1H, br s, CH_2CH=), 5.82 (1H, d, *J* = 15.5 Hz, CH=*CH*CO), 6.99–7.14 (1H, m, *CH*=CHCO). The ethyl ester of **2a** was prepared in 92% yield as per literature method[30]. Anal. Calcd for $C_{14}H_{24}O_2$: C, 74.95; H, 10.78%. Found: C, 74.98; H, 10.81%. MS (%) M^+ at *m*/*z* 224 (82), 193 (27), 188 (17), 161 (100), 117 (27), 106 (25), (51). IR (neat). 3386, 2962, 2924, 1720, 1652, 1437, 1378, 1310, 1266, 1182, 1119, 1045, 695, 541 cm^{-1}. ^1H NMR major isomer: δ 0.95 (3H, d, *J* = 6.6 Hz, CHCH_3), 1.09–1.13 (6H, m, CH_3CHCH_2 and OCH_2CH_3), 1.60 and 1.69 (3H each, s, =C(CH_3)$_2$), 1.96–2.25 (4H, m, CH_2CHCH_2), 4.19 (2H, q, OCH_2), 5.08 (1H, br s, CH_2CH=), 5.82 (1H, d, *J* = 15.5 Hz, CH=*CH*CO), 6.94–6.99 (1H, m, *CH*=CHCO).

4.2.17. Preparation of 5,9-dimethyl-deca-2,8-dienoic acid piperidide (17)

This was prepared in 87% yield from **2a** by the procedure as described for compound **3**. Anal. Calcd for $C_{17}H_{29}NO$: C, 77.51; H, 11.10; N, 5.32%. Found: C, 77.53; H, 11.16; N, 5.38%. MS (%) M^{+1} at *m*/*z* M^++1 264 (100), 248 (35), 208 (20), 195 (18), 181 (25), 180 (40), 114 (20), 111 (18), 86 (35), 84 (76). IR (neat). 2928, 2855, 1656, 1620, 1440, 1253, 1220, 1137, 1015, 852, 554 cm^{-1}. ^1H NMR major isomer: δ 0.94 (3H, d, *J* = 6.9 Hz, CHCH_3), 1.08–1.16 (3H, m, CH_3CHCH_2), 1.25–1.70 (12H, m, =C(CH_3)$_2$ and –N–CH_2(CH_2)$_3$), 1.96–2.25 (4H, m, CH_2CHCH_2), 3.48–3.57 (4H, m, 4.19 –N(CH_2)$_2$), 5.08 (1H, br s, CH_2CH=), 6.26 (1H, d, *J* = 15.0 Hz, CH=*CH*CO), 6.76–6.84 (1H, m, *CH*=CHCO).

4.2.18. Preparation of 5,9-dimethyl-deca-2,8-dienoic acid benzyl amide (18)

This was prepared in 92% yield from **2a** by the procedure as described for compound **3**. Anal. Calcd for $C_{19}H_{27}NO$: C, 79.95; H, 9.53; N, 4.91. Found: C, 80.00; H, 9.56; N, 4.95. MS (%) M^+ at *m*/*z* 285 (12), 270 (28), 242 (46), 151 (75), 108 (100), 80 (20). IR (neat). 3292, 3065, 2923, 2853, 1666, 1632, 1547, 1454, 1376, 1240, 1095, 985, 741, 697 cm^{-1}. ^1H NMR major isomer: δ 0.92 (3H, d, *J* = 6.6 Hz, CHCH_3), 1.19–1.35 (3H, m, CH_3CHCH_2), 1.57 and 1.66 (3H each, s, =C(CH_3)$_2$), 1.98–2.08 (4H, m, CH_2CHCH_2), 4.42 (2H, s, –NHCH_2), 4.89 (1H, br s, CH_2CH=), 5.99 (1H, d, *J* = 15.13 Hz, CH=*CH*CO), 6.76–6.84 (1H, m, *CH*=CHCO), 7.20–7.13 (5H, m, 5× Ar-H).

4.2.19. Preparation of 5,9-dimethyl-deca-2,8-dienoic acid *n*-octylamide (19)

This was prepared in 87% yield from **2a** by the procedure as described for compound **3**. Anal. Calcd for $C_{20}H_{37}NO$: C, 78.11; H, 12.13; N, 4.55%. Found: C, 78.19; H, 12.16; N, 4.58%. MS (%) M^+ at *m*/*z* M^+ 307 (32), 265 (17), 224 (100), 206 (8), 181 (10), 156 (41), 141 (5), 132 (8), 94 (70). IR (neat). 3292, 2957, 2925, 2855, 1666, 1629, 1549, 1458, 1377, 1169, 980, 840, 722 cm^{-1}. ^1H NMR major isomer: δ 0.91–0.97 (6H, m, CHCH_3 and CH_2CH_3), 1.18–1.26 (13H, m, CH_3CHCH_2 and –NHCH$_2$CH$_2$(CH_2)$_5$), 1.38–1.41 (2H, m, –NHCH$_2$CH_2), 1.57 and 1.67 (3H each, s, =C(CH_3)$_2$), 1.97–2.18 (4H, m, CH_2CHCH_2), 3.29 (2H, m, –NHCH_2), 5.15 (1H, br s, CH_2CH=), 5.74 (1H, d, *J* = 15.2 Hz, CH=*CH*CO), 6.72–6.87 (1H, m, *CH*=CHCO).

4.2.20. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid amide of D-2-aminobutanol (20)

This was prepared in 77% yield from **2a** by the procedure as described for compound **3**. Anal. Calcd for $C_{16}H_{29}NO_2$: C, 71.22; H, 10.93; N, 5.24%. Found: C, 71.24; H, 10.96; N, 5.29%. MS (%) M^+ at *m*/*z* 267 (5), 249 (15), 234 (20), 206 (5), 192 (21), 178 (100), 151 (10), 124 (20), 109 (15). IR (neat). 3289, 2963, 2925, 1722, 1632, 1546, 1460, 1378, 1263, 1095, 982, 671 cm^{-1}. ^1H NMR major isomer: δ 0.89–1.07 (6H, m, CHC*H*$_3$ and CH_2CH_3), 1.25–1.41 (5H, m, CH_3CHCH_2 and –NHCHC*H*$_2$), 1.60 and 1.68 (3H each, s, =C(C*H*$_3$)$_2$), 1.97–1.99 (4H, m, C*H*$_2$CHC*H*$_2$), 3.36–3.39 (1H, m, –NHC*H*), 3.69 (2H, br s, CHC*H*$_2$OH), 5.08 (1H, br s, CH_2CH=), 5.82 (1H, d, *J* = 15.5 Hz, CH=C*H*CO), 6.96–7.01 (1H, m, C*H*=CHCO).

4.2.21. Preparation of 5,9-dimethyl-deca-2,4,8-trienoic acid amide of L-2-aminobutanol (21)

This was prepared in 88% yield from **2a** by the procedure as described for compound **3**. Anal. Calcd for $C_{16}H_{29}NO_2$: C, 71.24; H, 10.96; N, 5.28%. Found: C, 71.28; H, 10.98; N, 5.30%. MS (%) M^+ at *m*/*z* 267 (15), 249 (18), 234 (28), 206 (34), 191 (20), 178 (100), 151 (15), 124 (10), 109 (18). IR (neat). 3293, 2969, 2925, 1719, 1632, 1540, 1469, 1372, 1265, 1095, 1043, 1011, 982, 671 cm^{-1}. ^1H NMR major isomer: δ 0.86–1.09 (6H, m, CHC*H*$_3$ and CH_2CH_3), 1.29–1.46 (5H, m, $CH_3CHCH_2$and –NHCHC*H*$_2$), 1.61 and 1.69 (3H each, s, =C(C*H*$_3$)$_2$), 1.98–2.02 (4H, m, C*H*$_2$CHC*H*$_2$), 3.41–3.50 (1H, m, –NHC*H*), 3.71 (2H, br s, CHC*H*$_2$OH), 5.11 (1H, br s, CH_2CH=), 5.85 (1H, d, *J* = 15.5 Hz, CH=C*H*CO), 6.98–7.05 (1H, m, C*H*=CHCO).

4.2.22. Preparation of 5,9-dimethyl-deca-2,8-dienoic acid *o*-anisidine (22)

This was prepared in 97% yield from **2a** by the procedure as described for compound **3**. Anal. Calcd for $C_{19}H_{27}NO_2$: C, 75.71; H, 9.03; N, 4.65%. Found: C, 75.73; H, 9.05; N, 4.69%. MS (%) M^+ at *m*/*z* 301 (25), 286 (20), 270 (32), 243 (12), 200 (15), 188 (13), 152 (15), 123 (100), 110 (27), 97 (26). IR (neat). 2957, 2924, 2853, 1686, 1643, 1601, 1524, 1460, 1377, 1219, 978, 746, 599 cm^{-1}. ^1H NMR major isomer: δ 0.94 (3H, d, *J* = 6.8 Hz, CHC*H*$_3$), 1.25–1.39 (3H, m, CH_3CHCH_2), 1.59 and 1.64 (3H each, s, =C(C*H*$_3$)$_2$), 2.01–2.22 (4H, m, C*H*$_2$CHC*H*$_2$), 3.86 (3H, s, OC*H*$_3$), 5.08 (1H, br s, CH_2CH=), 5.96 (1H, d, *J* = 14.15 Hz, CH=C*H*CO), 6.85–7.05 (1H, m, C*H*=CHCO).

4.2.23. Preparation of 9-formyl-5-methyl-deca-2,6,8-trienoic acid piperidide (23)

The amide **3** (0.2 g, 0.76 mmol) was dissolved in a mixture of acetic acid and water (9:1), added selenium dioxide (10 mg, 0.76 mmol) and stirred the contents for 6 h at 20 °C. The reaction mixture was diluted with ice-cold water and extracted with ethyl acetate (2× 100 mL). The organic layer washed with water (2× 25 mL), dried over anhydrous sodium sulfate and concentrated on rotavapour under reduced pressure to give crude product, which was purified on silica gel column using pet.ether/ethyl acetate (70:30) as an eluent to give compound **23** (0.15 g, yield 71%). Anal. Calcd for $C_{17}H_{25}NO_2$: C, 74.14; H, 9.15; N, 5.09%. Found: C, 74.16; H, 9.17; N, 5.17%. MS (%) M^+ at *m*/*z* 283 (28), 200 (68), 199 (61), 182 (22), 171 (31), 153 (58), 149 (60), 130 (54), 101 (100). IR (neat). 2935, 2856, 1684, 1644, 1442, 1271, 1136, 1020, 853 cm^{-1}. ^1H NMR major isomer: *δ* 1.59 (6H, br s, –NCH$_2$(C*H*$_2$)$_3$), 1.80 and 1.84 (3H each, s, C*H*$_3$CCHO and CC*H*$_3$), 2.10–2.25 (4H, m, 2× C*H*$_2$), 3.53 (4H, br s, –N–(C*H*$_2$)$_2$), 5.97 (1H, br s, CH_2CH=), 6.15 (1H, d, *J* = 14.3 Hz, CH=C*H*CO), 6.27 (1H, d, *J* = 12.4 Hz, C*H*CH=CHCO), 7.47 (1H, dd, *J* = 12.4 and 14.3 Hz, CHC*H*=CHCO), 9.31 (1H, s, C*H*O).

4.2.24. Preparation of 9-formyl-5-methyl-deca-2,6,8-trienoic acid benzyl amide (24)

This was prepared in 74% yield from compound **4** by the same procedure as described for compound **23**. Anal. Calcd for $C_{19}H_{23}NO_2$: C, 76.73; H, 7.80; N, 4.71%. Found: C, 76.79; H, 7.86; N, 4.73%. MS (%) M^+ at *m*/*z* 297 (26), 281 (100), 267 (28), 207 (35), 193 (24), 121 (25), 97 (40), 55 (35). IR (neat). 3660, 3317, 2924, 2853, 2361, 1657, 1545, 1448, 1279, 1094, 1022, 700 cm^{-1}. ^1H NMR major isomer: *δ* 1.74 and 1.91 (3H each, s, C*H*$_3$CCHO and CC*H*$_3$), 2.51 (4H, br s, 2× C*H*$_2$), 4.54 (2H, d, *J* = 5.6 Hz, NHC*H*$_2$), 5.79 (1H, d, *J* = 14.4 Hz, CH=C*H*CO), 6.01 (1H, d, *J* = 10.8 Hz, C*H*CH=CHCO), 6.44 (1H, br s, CH2C*H*=), 7.27–7.32 (5H, m, 5× Ar-H), 7.57 (1H, dd, *J* = 10.8 and 14.5 Hz, CHC*H*=CHCO), 9.39 (1H, s, CHO).

4.2.25. Preparation of 9-formyl-5-methyl-deca-2,6,8-trienoic acid *n*-octylamide (25)

This was prepared in 75% yield from compound **5** by the same procedure as described for compound **23**. Anal. Calcd for $C_{20}H_{33}NO_2$: C, 75.19; H, 10.41; N, 4.38%. Found: C, 75.21; H, 10.44; N, 4.40%. MS (%) M^+ at *m*/*z* M^++1 320 (8) 291 (56) 185 (61) 171 (100) 143 (51) 97 (60). IR (neat). 3272, 2925, 2855, 1654, 1549, 1465, 1376, 1033, 722 cm^{-1}. ^1H NMR major isomer: *δ* 0.88 (3H, t, *J* = 8.0 Hz, CH_2CH_3), 1.28 (6H, br s, -(C*H*$_2$)$_6$CH$_3$), 1.74 and 1.95 (3H each, s, C*H*$_3$CCHO and CC*H*$_3$), 2.21 (4H, br s, 2× C*H*$_2$), 3.31–3.42 (2H, m, NHC*H*$_2$), 5.85 (1H, d, *J* = 14.1 Hz, CH=C*H*CO), 6.03 (1H, d, *J* = 10.8, C*H*CH=CHCO), 6.35 (1H, br s, CH_2CH=), 7.52 (1H, dd, *J* = 10.8 and 14.1 Hz, CHC*H*=CHCO), 9.35 (1H, s, C*H*O).

4.2.26. Preparation of 9-formyl-5-methyl-deca-2,6,8-trienoic acid amide of D-2-aminobutanol (26)

This was prepared in 68% yield from compound **6** by the same procedure as described for compound **23**. Anal. Calcd for $C_{16}H_{25}NO_3$: C, 68.79; H, 9.02; N, 5.01%. Found: C, 68.81; H, 9.07; N, 5.06%. MS (%) M^+ at *m*/*z* M^++1 280 (2), 249 (100), 131 (51), 206 (55), 193 (74), 163 (60). IR (neat). 2924, 2853, 1725, 1655, 1459, 1377, 1050, 667 cm^{-1}. ^1H NMR major isomer: *δ* 0.85 (3H, t, *J* = 8.5 Hz, CH_2CH_3), 1.77 and 1.88 (3H each, s, C*H*$_3$CCHO and CC*H*$_3$), 2.17 (4H, br s, 2× C*H*$_2$), 3.68 (2H, br s, C*H*$_2$OH), 3.95 (1H, br s, NH–C*H*), 5.81 (1H, d, *J* = 14.9 Hz, CH=C*H*CO), 6.0 (1H, br s, CH_2CH=), 6.41 (1H, d, *J* = 11.2 Hz, C*H*CH=CHCO), 7.51 (1H, dd, *J* = 11.2; 14.9 Hz, CHC*H*=CHCO), 9.38 (1H, s, C*H*O).

4.2.27. Preparation of 9-formyl-5-methyl-deca-2,6,8-trienoic acid amide of L-2-aminobutanol (27)

This was prepared in 70% yield from compound **7** by the same procedure as described for compound **23**. Anal. Calcd for $C_{16}H_{25}NO_3$: C, 68.63; H, 9.00; N, 5.09%. Found: C, 68.67; H, 9.07; N, 5.12%. MS (%) M^+ at *m*/*z* M^++1 280 (27), 249 (10), 131 (100), 206 (12), 193 (79), 163 (45). IR (neat). 2920, 2867, 1725, 1650, 1440, 1371, 1055, 660 cm^{-1}. ^1H NMR major isomer: *δ* 0.87 (3H, t, *J* = 8.5 Hz, CH_2CH_3), 1.77 and 1.88 (3H each, s, C*H*$_3$CCHO and CC*H*$_3$), 2.17 (4H, br s, 2× C*H*$_2$), 3.68 (2H, br s, C*H*$_2$OH), 3.95 (1H, br s, NH–C*H*), 5.81 (1H, d, *J* = 14.9 Hz, CH=C*H*CO), 6.0 (1H, br s, CH_2CH=), 6.41 (1H, d, *J* = 11.2 Hz, C*H*CH=CHCO), 7.51 (1H, dd, *J* = 11.2 and 14.9 Hz, CHC*H*=CHCO), 9.38 (1H, s, C*H*O).

4.2.28. Preparation of 9-formyl-5-methyl-deca-2,6,8-trienoic acid pyrrolidide (28)

This was prepared in 78% yield from compound **11** by the same procedure as described for compound **23**. Anal. Calcd for $C_{16}H_{23}NO_2$: C, 73.53; H, 8.87; N, 5.35%. Found: C, 73.55; H, 8.89; N, 5.39%. MS (%) M^+ at *m*/*z* 262 (15), 246 (10), 232 (18), 208 (93), 192 (28), 161 (95), 149 (100) and 137 (55). IR (neat). 2958, 2925, 2854, 1684, 1645, 1438, 1259, 1020, 801 cm^{-1}. ^1H NMR major iso-

mer: δ 1.56 (4H, br s, –N–$CH_2(CH_2)_2$), 1.74 and 1.87 (3H each, s, CH_3CCHO and CCH_3), 2.14–2.31 (4H, m, 2× CH_2), 3.47 (4H, br s, –N–$(CH_2)_2$), 5.95 (1H, br s, CH_2CH=), 6.18 (1H, d, J = 14.8 Hz, CH=CHCO), 6.29 (1H, d, J = 11.4 Hz, CHCH=CHCO), 7.50 (1H, dd, J = 11.4 and 14.8 Hz, CHCH=CHCO), 9.39 (1H, s, CHO).

4.2.29. Preparation of 9-formyl-5-methyl-deca-2,6,8-trienoic acid morpholide (29)

This was prepared in 74% yield from compound **14** by the same procedure as described for compound **23**. Anal. Calcd for $C_{16}H_{23}NO_2$: C, 69.29; H, 8.36; N, 5.05%. Found: C, 69.31; H, 8.38; N, 5.07%. MS (%) M^+ at m/z 277 (12), 248 (18), 204 (100), 176 (20), 162 (74), 132 (10), 97 (20). IR (neat). 2961, 2925, 2854, 1716, 1644, 1437, 1300, 1234, 1115, 1041, 757 cm^{-1}. ^1H NMR major isomer: δ 1.81 and 1.88 (3H each, s, CH_3CCHO and CCH_3), 2.42 (4H, br s 2× CH_2), 3.38 (4H, br s –N($CH_2)_2$), 3.71 (4H, br s –O–$(CH_2)_2$), 5.96 (1H, br s CH_2CH=), 6.21 (1H, d, J = 14.9 Hz, CH=CHCO), 6.37 (1H, d, J = 11.4 Hz, CHCH=CHCO), 7.51 (1H, dd, J = 11.2 and 14.9 Hz, CHCH=CHCO), 9.32 (1H, s, CHO).

4.2.30. Preparation of 9-formyl-5-methyl-deca-2,4,8-trienoic acid diisopropylamide (30)

This was prepared in 76% yield from compound **14** by the same procedure as described for compound **23**. Anal. Calcd for $C_{18}H_{29}NO_2$: C, 74.18; H, 10.03; N, 4.81%. Found: C, 74.91; H, 10.06; N, 4.86%. MS (%) M^+ at m/z 295 (76), 268 (80), 253 (10), 211 (96), 169 (55), 154 (100), 126 (30), 101 (35), 85 (25). IR (neat). 2925, 2854, 1687, 1641, 1458, 1376, 1260, 1097, 801 cm^{-1}. ^1H NMR major isomer: δ 0.85 (12H, br s, 2× CH($CH_3)_2$), 1.85 and 1.90 (3H each, s, CH_3CCHO and CCH_3), 4.05 (2H, m, 2× CH$(CH_3)_2$), 2.35–2.55 (4H, m, 2× CH_2), 6.01 (1H, br s CH_2CH=), 6.25 (1H, d, J = 14.1 Hz, CH=CHCO), 6.45 (1H, d, J = 11.4 Hz, CHCH=CHCO), 7.53 (1H, dd, J = 11.4; 14.1 Hz, CHCH=CHCO), 9.89 (1H, s, CHO).

4.2.31. Preparation of 9-formyl-5-methyl-deca-2,8-dienoic acid *o*-anisidine (31)

This was prepared in 72% yield from compound **22** by the procedure as described for compound **23**. Anal. Calcd for $C_{19}H_{25}NO_3$: C, 72.36; H, 7.99; N, 4.44%. Found: C, 72.39; H, 7.00; N, 4.48%. MS (%) M^+ at m/z 315 (25), 287 (100), 256 (32), 179 (12), 164 (15), 136 (13), 110 (15), 95 (40), 69 (27). IR (neat). 2957, 2924, 2853, 1686, 1643, 1601, 1524, 1460, 1377, 1219, 978, 746, 599 cm^{-1}. ^1H NMR: δ 0.92 (3H, d, J = 6.9 Hz, CHCH_3), 1.26–1.35 (3H, m, CH_3CHCH_2), 1.95 (3H, s, CH_3CCHO), 2.21–2.32 (4H, m, CH_2CHCH_2), 3.89 (3H, s, OCH_3), 6.01 (1H, d, J = 14.15 Hz, CH=CHCO),6.18 (1H, br s CH_2CH=), 6.86–7.05 (5H, m, 4× Ar-H and CH=CHCO), 9.49 (1H, s, CHO).

4.2.32. Preparation of 9-formyl 5-dimethyl-deca-2,8-dienoic acid *n*-octylamide (32)

This was prepared in 78% yield from compound **19** by the same procedure as described for compound **23**. Anal. Calcd for $C_{20}H_{35}NO_2$: C, 74.72; H, 10.97; N, 4.36%. Found: C, 74.74; H, 10.99; N, 4.31%. MS (%) M^+ at m/z M^+ 307 (32), 265 (17), 224 (100), 206 (8), 181 (10), 156 (41), 141 (5), 132 (8), 94 (70). IR (neat). 3292, 2957, 2925, 2855, 1666, 1629, 1549, 1458, 1377, 1169, 980, 840, 722 cm^{-1}. ^1H NMR major isomer: δ 0.91–0.97 (6H, m, CHCH_3 and CH_2CH_3), 1.18–1.26 (13H, m, CH_3CHCH_2 and –NHC$H_2CH_2(CH_2)_5$), 1.38–1.41 (4H, m, –NH($CH_2)_2$), 1.57 and 1.67 (3H each, s, =C($CH_3)_2$), 1.97–2.18 (4H, m, CH_2CHCH_2), 3.29 (2H, m, –NHCH_2), 5.15 (1H, br s CH_2CH=), 5.74 (1H, d, J = 15.2 Hz, CH=CHCO), 6.72–6.87 (1H, m, CH=CHCO).

4.2.33. Preparation of 9-formyl-5-methyl-deca-2,8-dienoic acid amide of D-2-aminobutanol amide (33)

This was prepared in 77% yield from compound **20** by the same procedure as described for compound **23**. Anal. Calcd for $C_{16}H_{27}NO_3$: C, 68.29; H, 9.67; N, 4.98%. Found: C, 68.32; H, 9.70; N, 5.02%. MS (%) M^++1 at m/z 280 (2), 249 (100), 131 (51), 206 (55), 193 (74), 163 (60). IR (neat). 2924, 2853, 1725, 1655, 1459, 1377, 1050, 667 cm^{-1}. ^1H NMR major isomer: δ 0.96–1.06 (6H, m, CHCH_3 and CH_2CH_3), 1.25–1.41 (5H, m, CH_3CHCH_2 and –NHCHCH_2), 1.60 (3H, s, =C(CH_3)CHO), 1.92–1.96 (4H, m, CH_2CHCH_2), 3.59–3.67 (1H, m, –NHCH), 3.74 (2H, br s $CHCH_2$OH), 6.26 (1H, d, J = 15.5 Hz, CH=CHCO), 6.39 (1H, br s CH_2CH=), 6.88–6.96 (1H, m, CH=CHCO), 9.33 (1H, s, CCHO).

4.2.34. Preparation of 9-formyl-5-methyl-deca-8-enoic acid piperidide (34)

A mixture of methanolic solution of amide **23** (65 mg) and 5% Pdc (20 mg) was hydrogenated at 22 psi for 4 h and the contents were filtered, washed with excess of methanol (5× 10 mL) and concentrated on a rotavapour at reduced pressure to give crude product which on CC over silica gel and elution with pet.ether/ethyl acetate (85:15) afforded compound **34** (18 mg, yield 25%) as a gummy mass. MS (%) M^+ at m/z 279 (7), 249 (11), 214 (14), 195 (30), 165 (100), 111 (43), 84 (33). IR (neat). 2917, 1683, 1648, 1446, 1406, 1240, 1133, 1010 cm^{-1}. ^1H NMR: δ 0.97 (3H, d, J = 7.0 Hz, CHCH_3), 1.25– 1.53 (13H, m, 6× CH_2 and CHCH_3), 1.84 (3H, s, C=CH_3), 1.91–2.13 (4H, m, 2× CH_2), 3.19 (4H, m, –N–$(CH_2)_2$), 5.71 (1H, br s CH_2CH=), 9.21 (1H, s, CHO).

4.2.35. Preparation of 9-formyl-5-methyl-deca-8-enoic acid benzyl amide (35)

This was prepared in 21% yield from compound **24** by the procedure described for compound **34** as a gummy mass. MS (%) M^+ at m/z 301 (3), 273 (12), 218 (12), 210 (40), 143 (11), 127 (100), 106 (33), 91 (49). IR (neat). 2940, 1681, 1651, 1546, 1453, 1352, 1243, 1111, 998 cm^{-1}. ^1H NMR: δ 0.99 (3H, d, J = 7.0 Hz, CHCH_3), 1.20–1.66 (7H, m, 3× CH_2 and CHCH_3), 1.81 (3H, s, C=CH_3), 1.88–2.19 (4H, m, 2× CH_2), 4.29 (2H, br s, –N–CH_2), 5.81 (1H, br s CH_2CH=), 6.99–7.18 (5h, m, 5× Ar-H), 9.17 (1H, s, CHO).

4.2.36. Preparation of 9-formyl-5-methyl-deca-8-enoic acid amide of D-2-aminobutanol (36)

This was prepared in 16% yield from compound **26** by the procedure described for compound **34** as a gummy mass. MS (%) M^+ at m/z 284 (5), 254 (11), 225 (13), 196 (31), 111 (100), 88 (51), 83 (12). IR (neat). 3344, 2937, 1678, 1643, 1534, 1434, 1244, 998 cm^{-1}. ^1H NMR: δ 0.89 (3H, d, J = 7.8 Hz, CH_2CH_3), 0.98 (3H, d, J = 6.93 Hz, CHCH_3), 1.28–1.81 (9H, m, 4× CH_2 and CHCH_3), 1.84 (3H, s, C=CH_3), 1.85–2.11 (4H, m, 2× CH_2), 3.66 (2H, br s CH_2OH), 3.91 (1H, m, –N–CH), 5.88 (1H, br s CH_2CH=), 9.11 (1H, s, CHO).

4.2.37. Preparation of 5,9-dimethyl-deca-8-enoic acid *n*-octyl amide (37)

This was prepared in 62% yield from compound **5** by the procedure described for compound **34** to gummy mass. MS (%) M^+ at m/z 309 (13), 294 (7), 254 (43), 240 (38), 196 (48), 127 (100), 113 (44). IR (neat). 3241, 2939, 2837, 1646, 1543, 1444, 1382, 1367, 1068, 947 cm^{-1}. ^1H NMR: δ 0.94 (3H, t, J = 7.8 Hz, CH_2CH_3), 0.98 (3H, d, J = 7.1 Hz, CHCH_3), 1.18–1.76 (19H, m, 9× CH_2 and CHCH_3), 1.62 and 1.64 (3H each, s, =C($CH_3)_2$), 1.88–2.17 (4H, m, 2× CH_2), 3.31 (2H, m, –NHCH_2), 5.21 (1H, br s CH_2CH=).

4.2.38. Preparation of 5,9-dimethyl-deca-8-enoic acid *o*-anisidine amide (38)

This was prepared in 62% yield from compound **8** by the procedure described for compound **34** as a gummy mass. MS (%) M^+ at m/z 313 (6), 283 (21), 258 (33), 244 (15), 228 (12), 214 (21), 206 (17), 137 (37), 107 (100). IR (neat). 2942, 2912, 1653, 1533, 1455, 1385, 1366, 1212, 989 cm^{-1}. ^1H NMR: δ 0.97 (3H, d, J = 7.0 Hz, CHCH_3), 1.28–1.62 (7H, m, 3× CH_2 and CHCH_3), 1.65

and 1.67 (3H each, s, =C(CH_3)$_2$), 1.84–2.14 (4H, m, 2× CH_2), 5.10 (1H, br s CH_2CH=), 6.64–7.07 (4H, m, 4× Ar-H=C(CH_3)$_2$).

4.3. Pharmacology

4.3.1. Determination of minimum effective concentration (MEC) of the EPIs

The MIC of ciprofloxacin was determined against *S. aureus* SA1199 and *S. aureus* SA1199B in Muller–Hinton broth in the presence of increasing amounts of efflux pump inhibitors by broth checkerboard synergy method in a microtitre plates with twofold serial dilutions.[50] Each candidate EPI was tested at seven concentrations (100 to 1.56 μg/mL), and ciprofloxacin was tested at 10 concentrations (16 to 0.03 μg/mL). The plates were incubated for 18 h at 37 °C, and the wells were assessed visually for growth. The minimal effective concentration (MEC) was determined to be the minimal concentration of EPI that produced the maximal reduction in substrate MIC. No further decrease in substrate MIC was observed at EPI concentrations greater than the MEC.[55]

4.3.2. Efflux studies

Ethidium bromide accumulation and its efflux were determined from the active cells by reported method.[56] *S. aureus* SA 1199B was grown overnight on Trypticase Soya Agar. Bacterial suspension (0.2 OD_{550}) was prepared in uptake buffer (NaCl, 110 mM; KCl, 7 mM; NH_4Cl, 50 mM; Na_2HPO_4, 0.4 mM; Tris base, 52 mM; glucose, 0.2%, adjusted the solution to pH 7.5 with HCl). The suspensions were exposed to 2 μg/mL ethidium bromide in the presence of the most active EPI (**26** at 6.25 μg/mL) and piperine (at 50 μg/mL), respectively, for 30 min at 37 °C. The cells were pelleted down by centrifugation and resuspended in fresh buffer. The loss of fluorescence was recorded for 30 min at 5 min interval at excitation and emission wavelengths of 530 and 600 nm in a spectrophotometer (Perkin-Elmer model LS50).

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